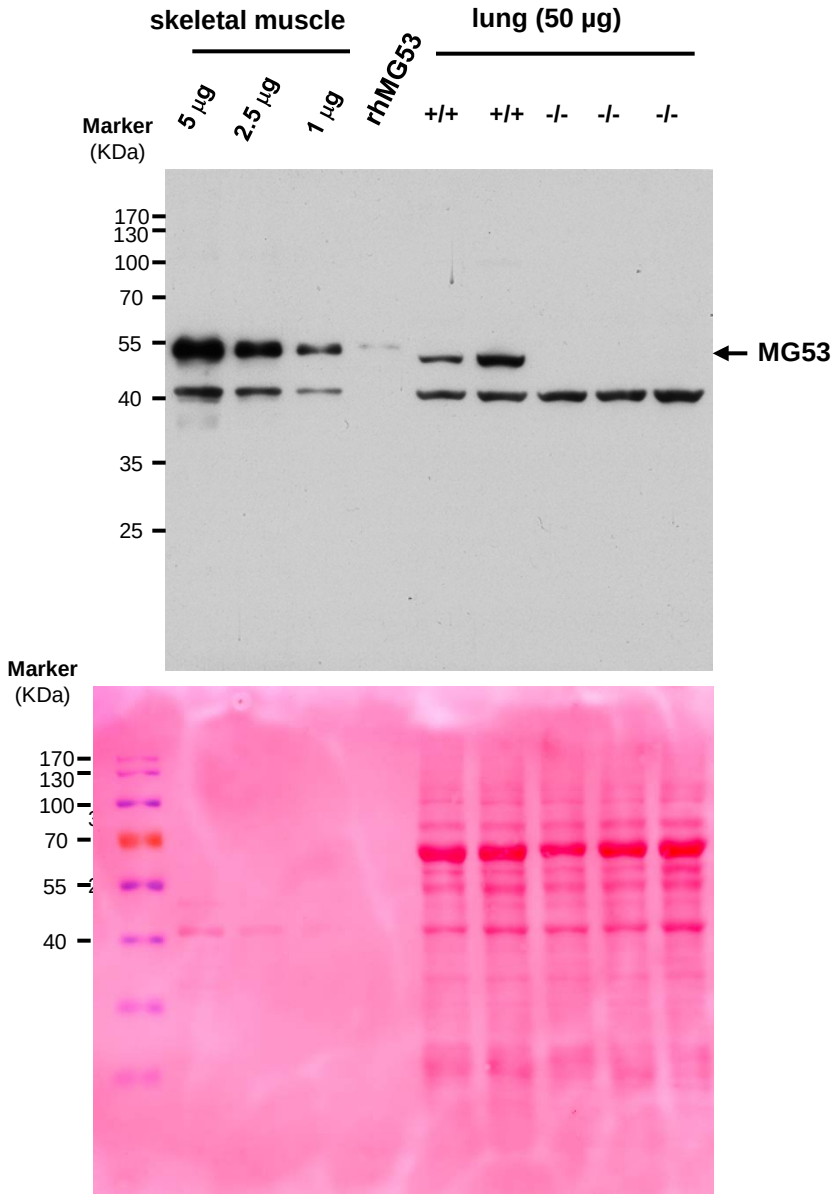
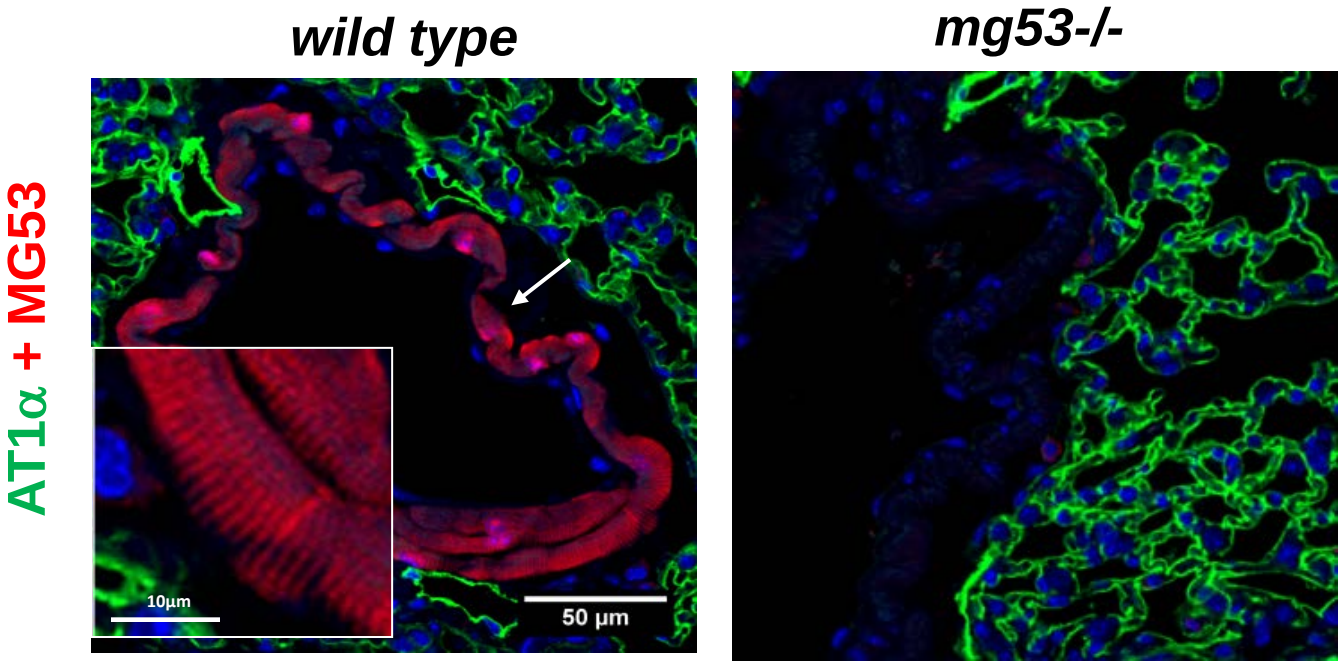
Supplementary Information

Supplementary Figure 1



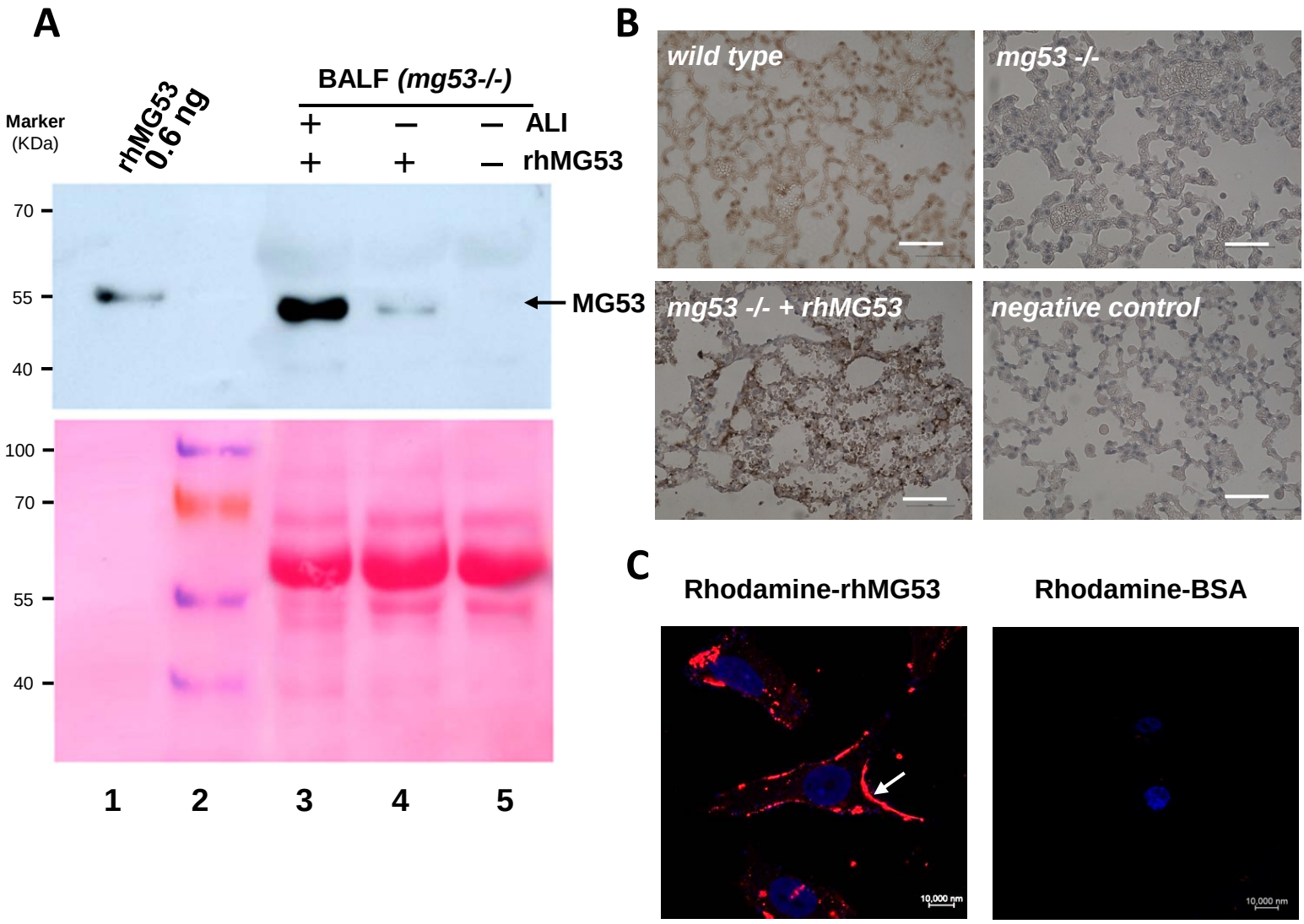
Supplementary Figure 1. The full scale images of Figure 1A.

Supplementary Figure 2



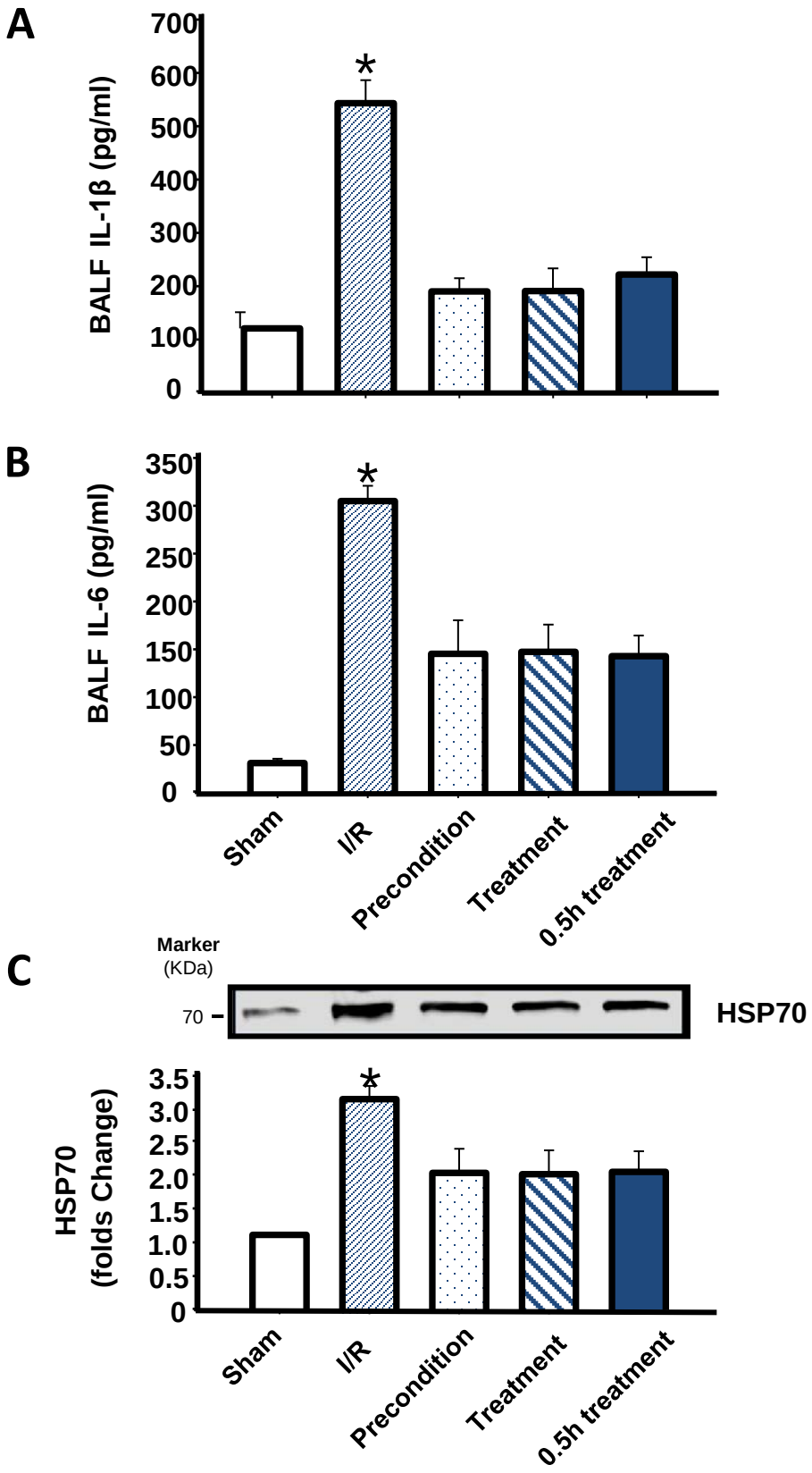
Supplementary Figure 2. Immuno-histochemical staining of lung from *wild type* or *mg53-/-* mice. Immunostaining protocol was the same in Fig. 1F. In a small portion of staining with the pulmonary vessel wall, strong MG53 staining was observed. High magnification image shown as insert (left panel) revealed striation pattern for MG53, suggestive of cardiomyocyte origin. This observation is consistent with a recent report by J-P. Jin and colleagues who demonstrated that the lung tissue from mouse and rat contain venous cardiomyocytes (Krachlauer et al (2013) FEBS Journal 280: 880-891).

Supplementary Figure 3



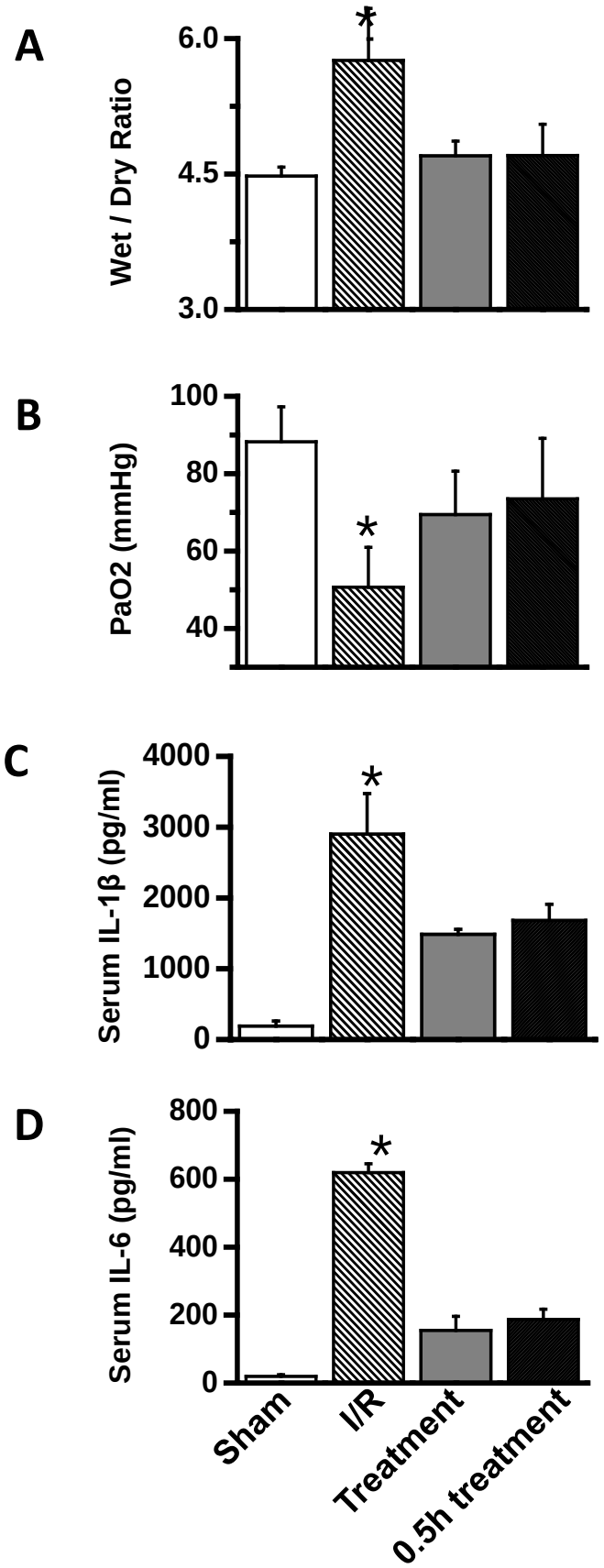
Supplementary Figure 3. (A) SDS-PAGE and immunoblot of rhMG53 from BALF derived from *mg53-/-* mice. The *mg53-/-* mice received intravenous injection of rhMG53 (2 mg/kg), and then underwent ventilation-induced acute lung injury (ALI) (*lane 3*). *mg53-/-* mice receiving saline injection was used as control (*lane 5*). BALF (20 μl) were collected and analyzed. Compared with mice receiving rhMG53 that were not subjected to ALI (*lane 4*), high level of rhMG53 was detected in BALF from animals subjected to ALI (*lane 3*). Recombinant rhMG53 was loaded as a reference control. (**B**) IHC staining of lung tissues from *wild type* (upper left), *mg53-/-* (upper right), *mg53-/-* mice treated with rhMG53 (2 mg/kg) (lower left) and IgG negative staining control (lower right). A custom made rabbit monoclonal anti-MG53 antibody was used for immunostaining and signal detected using the Polink-2 plus Polymer HRP kit (GBI Labs, Mukilteo, WA). The studies were repeated at least three times. Scale bars represent 50 μm. (**C**) Vascular endothelial cells (VECs) were induced with A/R (anoxia/reoxygenation) injury by 2 hour-anoxia pretreatment and followed by incubation with Rhodamine labeled-MG53 or BSA during the 2 hour- reoxygenation period. Confocal imaging revealed plasma membrane labeling of Rhodamine-rhMG53 (arrow), whereas such membrane attachment was not detected when cells were treated with Rhodamine-labeled BSA.

Supplementary Figure 4



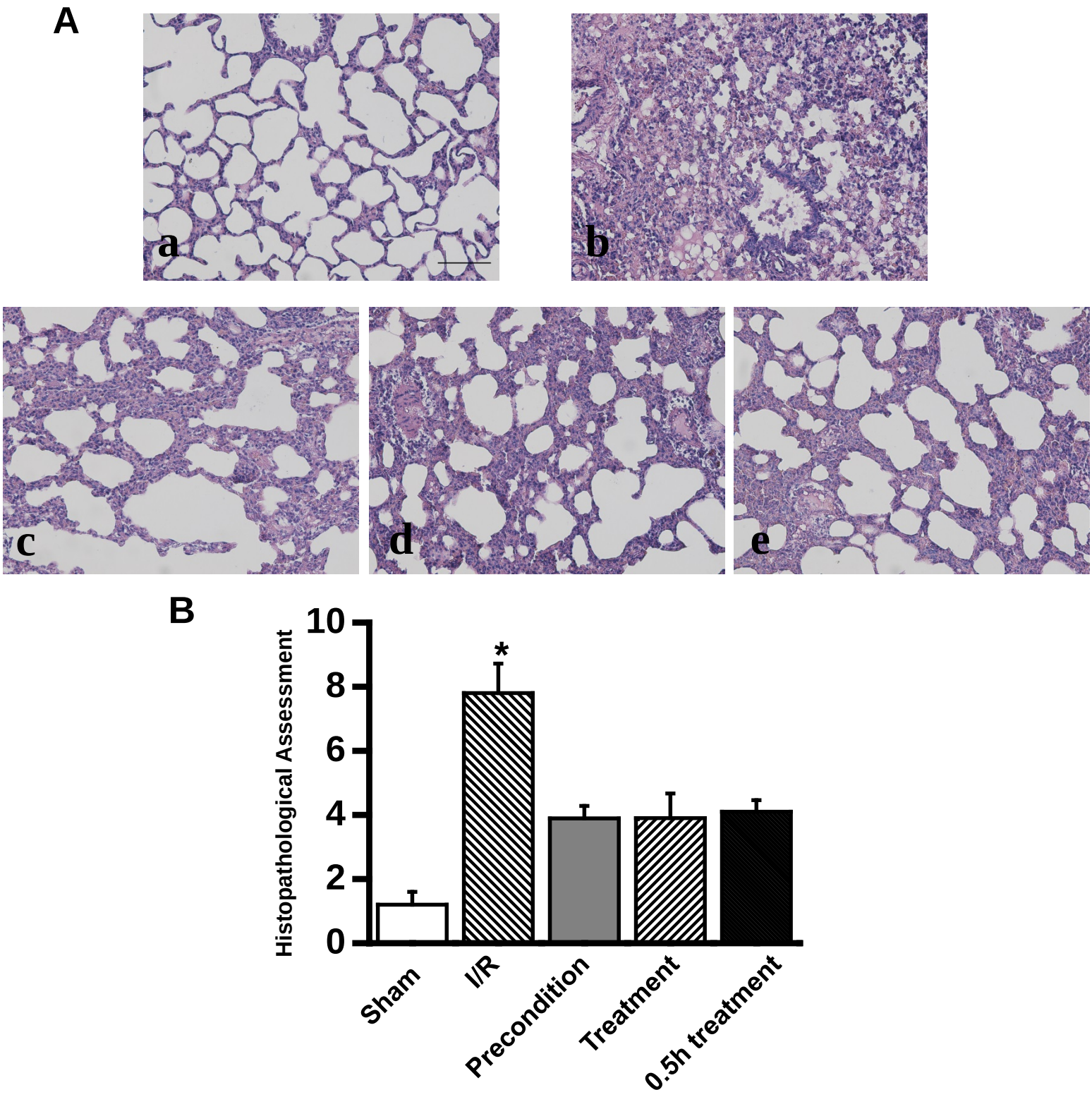
Supplementary Figure 4. Administration of rhMG53 improves damage-associated molecular patterns (DAMP) in bronchial alveolar lavage fluid (BALF) derived from rats subjected to I/R-induced ALI. IL-1β (**A**) and IL-6 (**B**) levels in BALF were determined using ELISA. (**C**) HSP70 (Heat Shock Protein 70) (anti-HSP70, Thermo Scientific-Pierce MA3-006) was measured with immmunoblot with 20 μl of BALF per lane from each condition. Intravenous delivery of rhMG53 before ischemia (precondition), immediately after ischemia (treatment), or 0.5 h after reperfusion (0.5 h treatment) all led to reduction of IL-1β, IL-6, and HSP70 in BALF. * P<0.05 vs. others (n=5), ANOVA, mean ± SEM.

Supplementary Figure 5



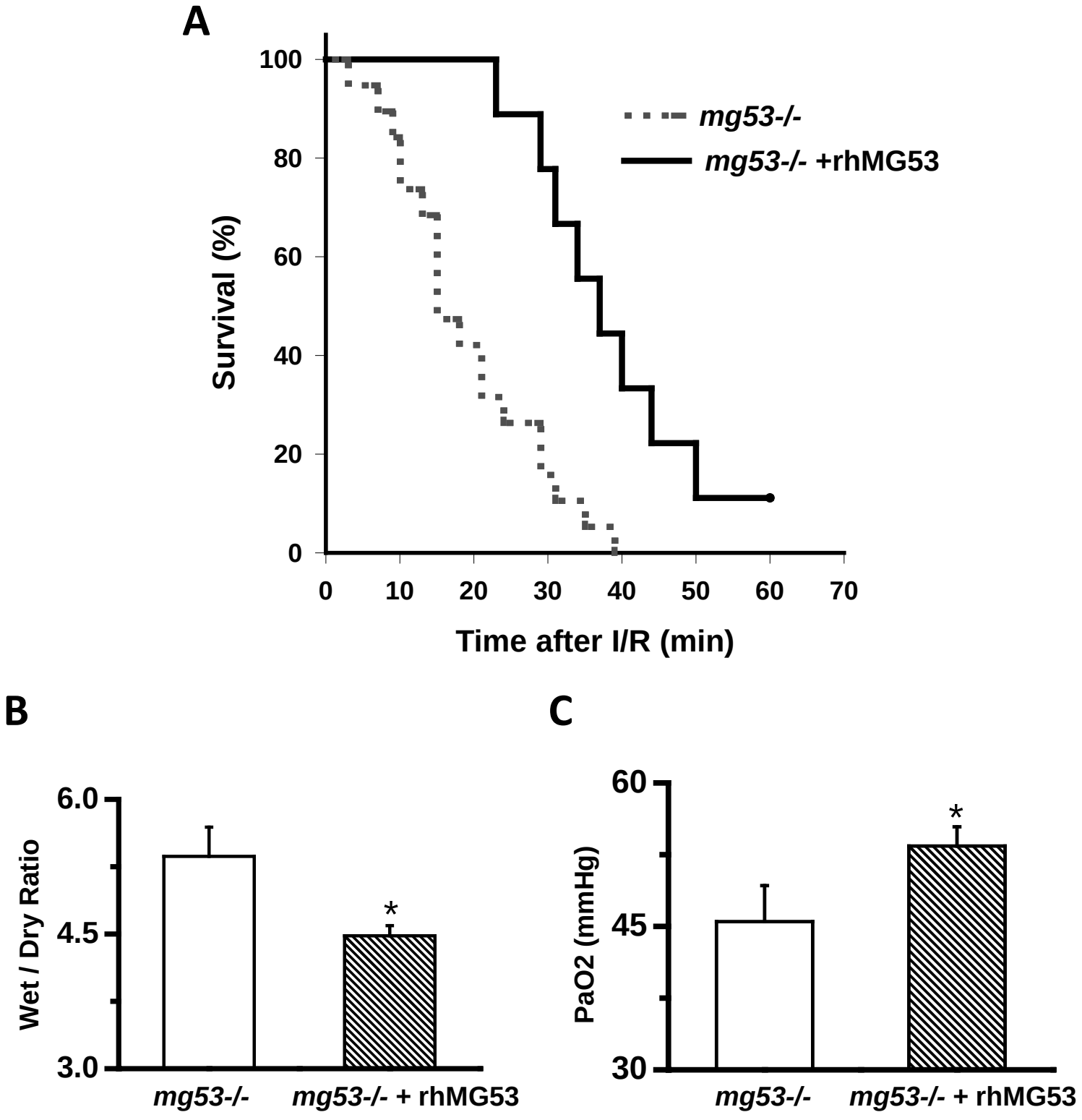
Supplementary Figure 5. The protective effect of rhMG53 against administration immediately after ischemia (treatment) or 0.5 h after reperfusion (0.5h treatment) on I/R induced lung injury in rats. Under both conditions, rats receiving treatment of rhMG53 showed amelioration of lung edema as reflected by the reduced wet/dry ratio (**A,** *P<0.05 vs. others, n=5 per group, ANOVA); improvement of gas exchange function as reflected by the restoration of plasma PaO2 concentrations (**B,** *P<0.05 vs. others, n=6 in each group, ANOVA). Administration of rhMG53 also led to reduction of serum IL-1β (**C**) and IL-6 (**D**) in I/R injured rats (*P<0.05 vs. others, n=5, ANOVA). All data represent mean ± SEM. These results suggest that intravenous delivery of rhMG53 has beneficial effect in treatment of ALI in rat model.

Supplementary Figure 6



Supplementary Figure 6. Histopathological changes of lung in I/R rats with or without rhMG53 administration. **A.** Representative H/E staining of lung sections were shown in sham group (**a**), I/R induced ALI (**b**), rhMG53 administered 30 min prior to ischemia (precondition) (**c**), rhMG53 administered immediately after ischemia (treatment) (**d**), or rhMG53 administered 0.5 hour after reperfusion (0.5h treatment) (**e**). Scale bar = 100 μm. **B.** Histopathological assessments were conducted in a blinded fashion using the grading criteria listed in Table 2. Statistical analyses showed that rhMG53 administration under the three different conditions all led to improvement of pathological changes of ALI (*P<0.05 vs. others, ANOVA, mean ± SEM).

Supplementary Figure 7



Supplementary Figure 7. Effect of rhMG53 administration on *mg53-/-* mice. **A**. Survival rate of *mg53-/-* mice and *mg53-/-* mice treated with rhMG53 was recorded after I/R. The product limit (Kaplan-Meier) estimate of the cumulative survival was assessed with the log-rank test to evaluate for significant differences in survival (*P<0.05 vs. rhMG53 untreated mice; n=19 for *mg53-/-* mice and n=9 for *mg53-/-* mice treated with rhMG53). **B**. Lung edema was measured as the wet/dry weight ratio of the excised lung tissue from mice (*P<0.05 vs. rhMG53 untreated mice, n=5 in each group, ANOVA, mean ± SEM). **C**. Arterial blood samples were drawn from individual mice, the plasma PaO2 concentrations were measured (*P<0.05 vs. rhMG53 untreated , n=6 in each group, ANOVA, mean ± SEM).

Supplementary Table 1

primer name	primer sequence	
AP1	CCATCCTAATACGACTCACTATAGGGC	Marathon-Ready cDNA adaptor
MA1	GTGATCCCACCGCCTTCAGGACCAG	5'-RACE primer
MS1	GCAGCTGTTCGATGCGCCAGTGACG	3'-RACE primer
clone name	clone sequence	
Clone #1~11	Full length coding sequence of MG53 starting at 5'-untranslated region	Products of 5'-RACE
Clone 12	Partial sequence of MG53 starting at 27th amino acid	Products of 5'-RACE
Clone 13, 14	Partial sequence of MG53 starting at 44th amino acid	Products of 5'-RACE
Clone 15	Partial sequence of MG53 starting at 52th amino acid	Products of 5'-RACE
Clone 16	Partial sequence of MG53 starting at 58th amino acid	Products of 5'-RACE
Clone 17,18	Full length coding sequence of MG53	Products of 3'-RACE

Supplementary Table 1. Primers used for RACE analysis of *mg53* in lung. A total of 16 randomly selected clones from 5' RACE product were sequenced. 11 clones were confirmed to start from 5' untranslated region with the complete same coding sequence of MG53 from muscle tissues. The other 5 clones started way into the coding region of muscle MG53, at the 27^{th} a.a., the 44^{th} a.a. ~, the 44^{th} a.a. ~, the 52^{nd} a.a. ~ and 58^{th} a.a.. Two independent 3' RACE clones were also sequenced and they had the same coding sequence of MG53 from muscle tissues.

Supplementary Table 2

	0	1	2	3
Peribronchial inflammatory cell infiltration	No	Prominent germinal centers of lymphoid follicules	Infiltration between lymphoid follicules	Confluent band like form
Alveolar septal infiltration	No	Minimal	Moderate	Severe, impending of lumen
Alveolar edema	No	Focal	In multiple alveoli	Widespread, Involving lobules
Alveolar exudate	No	Focal	In multiple alveoli	Prominent, widesp

Supplementary Table 2. The 4-point scale used for histopathologic assessment of I/R induced lung injury.